L2: Entry 13 of 28 File: USPT Feb 8, 2000

US-PAT-NO: 6022726
DOCUMENT-IDENTIFIER: US 6022726 A

TITLE: Genetically engineered attenuated viruses

DATE-ISSUED: February 8, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Palese; Peter	Leonia	NJ	07605	
Muster; Thomas	A-1190 Vienna			AT
Masayoshi; Enami	Kanazawa, Ishikawa 921			JP
Bergmann; Michael	New York	NY	10128	

US-CL-CURRENT: 435/236; 424/206.1, 435/320.1, 536/23.72, 536/24.2

CLAIMS:

What is claimed is:

1. An attenuated genetically engineered segmented RNA influenza virus containing at least one modified non-coding region comprising alterations to the stem structure of a promoter that down-regulates synthesis of at least the modified viral gene segment, so that at least some defective particles are produced during each round of viral replication in a host.

2. The attenuated virus of claim 1 in which the modified non-coding region down-regulates synthesis of a viral capsid gene.

3. The attenuated virus of claim 1 in which the modified non-coding region down-regulates synthesis of a viral envelope gene.

4. The attenuated virus of claim 1 in which the modified non-coding region down-regulates synthesis of a viral protease gene.

5. The attenuated virus of claim 1 in which the modified non-coding region down-regulates synthesis of a viral polymerase gene.

6. An attenuated genetically engineered influenza virus containing at least one modified non-coding region comprising alterations to the stem structure of a promoter that down-regulates transcription of at least the modified viral gene segment, so that the progeny virions produced during replication result in a subclinical level of infection in a host.

7. The attenuated virus of claim 6 in which the modified non-coding region down-regulates transcription of a viral capsid gene.

8. The attenuated virus of claim 6 in which the modified non-coding region down-regulates transcription of a viral envelope gene.

9. The attenuated virus of claim 6 in which the modified non-coding region down-regulates transcription of a viral protease gene.

10. The attenuated virus of claim 6 in which the modified non-coding region down-regulates transcription of a viral polymerase gene.

11. A method for generating an attenuated influenza virus of a first strain comprising the steps of:

(a) introducing a recombinant, negative strand RNA template, comprising at least one untranslated RNA sequence substituted with an untranslated RNA sequence containing the stem structure of a promoter from an influenza virus of a second strain wherein the stem structure is altered relative to that of the first strain, into a cell line including a helper virus capable of producing influenza virus RNA segments; and

(b) collecting virus from said cell line.

12. The method of claim 11, wherein said first strain is influenza A and said second strain is influenza B.

13. The method of claim 12, wherein said polymerase binding site is a part of said RNA sequence from influenza B virus.

14. The method of claim 11, wherein the introducing step further comprises introducing said template into said cell line as a ribonucleoprotein complex.

15. The method of claim 14, wherein said ribonucleoprotein complex contains influenza virus polymerase.

16. The method of claim 11, wherein said RNA sequence encoding polypeptide encodes influenza virus structural protein.

17. The method of claim 16, wherein said structural protein is NA.

18. The method of claim 11, wherein said RNA sequence encoding polypeptide encodes influenza virus polymerase.

19. Attenuated influenza virus obtained from the method of claim 11.

20. An attenuated influenza virus of a first strain comprising:

a sufficient number of single strand RNA segments of negative polarity, to generate an influenza virus within a host cell, wherein at least one of said RNA segments is a recombinant RNA template comprising at least one untranslated RNA sequence substituted with an untranslated RNA sequence containing the stem structure of a promoter from an influenza virus of a second strain, wherein the stem structure is altered relative to that of the first strain.

21. The virus of claim 20, wherein said first strain is influenza A and said second strain is influenza B.

22. The virus of claim 21, wherein said polypeptide is expressed from said RNA segment containing said untranslated RNA sequence from influenza B virus.

23. The virus of claim 21, wherein said virus contains two untranslated RNA sequences from influenza B virus.

24. The virus of claim 23, wherein said untranslated RNA sequences are located on influenza A virus segment 6.

25. The virus of claim 24, wherein said first untranslated RNA sequence is positioned at the 3' end of said segment.

26. The virus of claim 21, wherein said first untranslated RNA sequence contains a polymerase binding site.

27. The virus of claim 24, wherein said second untranslated RNA sequence from influenza B virus is positioned at the 5' end of said segment.

28. A pharmaceutical composition comprising the attenuated influenza virus of claim 20 in a pharmaceutically acceptable carrier.

29. An attenuated chimeric influenza virus containing an NA gene of influenza A virus, flanked by a noncoding region from influenza B virus.

30. The virus of claim 29, in which the noncoding region from influenza B virus is the NS polymerase binding site.

L2: Entry 14 of 28 File: USPT Dec 14, 1999

US-PAT-NO: 6001634
DOCUMENT-IDENTIFIER: US 6001634 A

TITLE: Recombinant negative strand RNA viruses

DATE-ISSUED: December 14, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Palese; Peter	Leonia	NJ	07605	
Garcia-Sastre; Adolfo	New York	NY	10029	

US-CL-CURRENT: 435/235.1; 435/320.1

CLAIMS:

What is claimed is:

1. A chimeric virus comprising influenza virus containing a heterologous RNA segment from another strain of influenza virus comprising the reverse complement of an mRNA coding sequence operatively linked to a binding site specific for an RNA-directed RNA polymerase of a negative strand RNA virus.

2. The chimeric virus of claim 1 in which the heterologous RNA segment is a hemaggutinin (HA) RNA segment from another strain of influenza virus.

3. The chimeric virus of claim 1 in which the heterologous RNA segment is a neuraminidase (NA) RNA segment from another strain of influenza virus.

4. A chimeric virus comprising influenza virus containing eight genomic segments from different strains of influenza virus, each of the segments comprising the reverse complement of an mRNA coding sequence operatively linked to a binding site specific for an RNA-directed RNA polymerase of a negative strand RNA virus.

5. The chimeric virus of claim 1 or claim 4 in which the binding site is specific for an RNA-directed RNA polymerase of an influenza virus.

6. The chimeric virus of claim 4 which contains HA and NA genomic segments from one strain of influenza virus and the remaining genomic segments from another strain of influenza virus.

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L2: Entry 17 of 28 File: USPT Dec 29, 1998

US-PAT-NO: 5854037
DOCUMENT-IDENTIFIER: US 5854037 A

TITLE: Recombinant negative strand RNA virus expression systems and vaccines

DATE-ISSUED: December 29, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Palese; Peter	Leonia	NJ		
Garcia-Sastre; Adolfo	New York	NY		

US-CL-CURRENT: 435/455; 435/235.1, 435/320.1, 435/456, 435/457, 435/69.1, 435/91.33, 530/350, 536/23.72

CLAIMS:

What is claimed is:

1. A recombinant RNA molecule comprising a binding site specific for an RNA-directed RNA polymerase of a negative strand RNA virus, operatively linked to a heterologous RNA sequence comprising the reverse complement of a bicistronic mRNA coding sequence containing an internal sequence that mediates internal initiation of translation.

2. The recombinant RNA molecule of claim 1 in which the polymerase binding site comprises the polymerase binding site contained in the 3'-noncoding flanking sequence of an internal genome RNA segment.

3. The recombinant RNA molecule of claim 1 in which the polymerase binding site comprises the terminal 12 nucleotides of the 3'-terminus of an influenza genomic segment.

4. The recombinant RNA molecule of claim 2 in which the 3'-noncoding flanking sequence comprises the sequence set forth as SEQ ID NO: 47: 5'-CCUGCUUUYGCU-3'.

5. A recombinant RNA molecule comprising a heterologous RNA sequence comprising the reverse complement of a bicistronic mRNA coding sequence containing an internal sequence that mediates internal initiation of translation, operatively linked to the 3'-noncoding flanking sequence of an influenza genomic RNA segment containing a viral polymerase binding site and operatively linked to the 5'-noncoding flanking sequence of an influenza genomic RNA sequence.

6. The recombinant RNA molecule of claim 5 in which the 5'-noncoding flanking sequence of an influenza genomic RNA sequence comprises the first 22 nucleotides of the 5'-terminus of an influenza genomic segment.

7. The recombinant RNA molecule of claim 5 in which the 5'-noncoding flanking sequence of an influenza RNA comprises the sequence set forth as SEQ ID NO: 48: 5'-AGUAGAAACAAGGGUGUUUUUU-3'.

8. A recombinant ribonucleoprotein (RNP) comprising the recombinant RNA molecule of claim 1 complexed with a purified RNA-directed RNA polymerase.

9. A recombinant RNP comprising the recombinant RNA molecule of claim 2 complexed with a purified influenza viral polymerase.

10. The recombinant RNP of claim 9 in which the purified influenza viral polymerase is obtained from RNPs fractionated by centrifugation on a CsCl gradient, wherein the purified influenza viral polymerase is isolated from the region of the gradient correlating to 1.5 to 2.0M CsCl.

11. A recombinant RNP comprising the recombinant RNA molecule of claim 5 complexed with a purified influenza viral polymerase.

12. The recombinant RNP of claim 11 in which the purified influenza viral polymerase is obtained from RNPs fractionated by centrifugation on a CsCl gradient, wherein the purified influenza viral polymerase is isolated from the region of the gradient correlating to 1.5 to 2.0M CsCl.

13. A chimeric virus comprising an influenza virus containing a heterologous RNA sequence comprising the reverse complement of a bicistronic mRNA coding sequence containing an internal sequence that mediates internal initiation of translation, operatively linked to an influenza viral polymerase binding site.

14. The chimeric virus of claim 13 in which the heterologous RNA sequence is contained within segment 1 of influenza virus.

15. The chimeric virus of claim 13 in which the heterologous RNA sequence is contained within segment 2 of influenza virus.

16. The chimeric virus of claim 13 in which the heterologous RNA sequence is contained within segment 3 of influenza virus.

17. The chimeric virus of claim 13 in which the heterologous RNA sequence is contained within segment 4 of influenza virus.

18. The chimeric virus of claim 13 in which the heterologous RNA sequence is contained within segment 5 of influenza virus.

19. The chimeric virus of claim 13 in which the heterologous RNA sequence is contained within segment 6 of influenza virus.

20. The chimeric virus of claim 13 in which the heterologous RNA sequence is contained within segment 7 of influenza virus.

21. The chimeric virus of claim 13 in which the heterologous RNA sequence is contained within segment 8 of influenza virus.

22. A chimeric virus comprising an influenza virus containing in addition to its eight genomic segments, an additional RNA segment containing a heterologous RNA sequence comprising the reverse complement of a bicistronic mRNA coding sequence containing an internal sequence that mediates internal initiation of translation, operatively linked to an influenza viral polymerase binding site.

23. The chimeric virus of claim 22, further containing a selectable coding sequence.

24. A chimeric virus comprising a negative-strand RNA virus containing a heterologous RNA sequence comprising the reverse complement of a bicistronic mRNA coding sequence containing an internal sequence that mediates internal initiation of translation, operatively linked to a polymerase binding site of the negative-strand RNA virus.

25. The chimeric virus of claim 24, further containing a selectable coding sequence.

26. A recombinant DNA molecule encoding the recombinant RNA molecule of claim 1

operatively linked to a transcription control element that binds a DNA-directed RNA polymerase.

27. A recombinant DNA molecule encoding the recombinant RNA molecule of claim 2 operatively linked to a transcription control element that binds a DNA-directed RNA polymerase.

28. A recombinant DNA molecule encoding the recombinant RNA molecule of claim 5 operatively linked to a transcription control element that binds a DNA-directed RNA polymerase.

29. A method for gene expression, comprising culturing a host cell transfected with the recombinant RNP of claim 8 whereby the heterologous RNA sequence is expressed.

30. A method for gene expression, comprising culturing a host cell transfected with the recombinant RNP of claim 9 whereby the heterologous RNA sequence is expressed.

31. A method for gene expression, comprising culturing a host cell transfected with the recombinant RNP of claim 11 whereby the heterologous RNA sequence is expressed.

32. A method for producing a chimeric negative-strand RNA virus, comprising culturing a host cell transfected with the recombinant RNP of claim 8 and infected with the parental strain of the negative-strand RNA virus, and recovering the chimeric virus from the resulting culture.

33. A method for producing a chimeric influenza virus, comprising culturing a host cell transfected with the recombinant RNP of claim 9 and infected with the parental strain of the influenza virus, and recovering the chimeric influenza virus from the resulting culture.

34. A method for producing a chimeric influenza virus, comprising culturing a host cell transfected with the recombinant RNP of claim 11 and infected with the parental strain of the influenza virus, and recovering the chimeric influenza from the resulting culture.

L2: Entry 18 of 28 File: USPT Nov 24, 1998

US-PAT-NO: 5840520
DOCUMENT-IDENTIFIER: US 5840520 A

TITLE: Recombinant negative strand RNA virus expression systems

DATE-ISSUED: November 24, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Clarke; David Kirkwood	Pacifica	CA		
Palese; Peter M.	Leonia	NJ		

US-CL-CURRENT: 435/69.1; 424/199.1, 435/235.1, 435/320.1, 536/23.1

CLAIMS:

What is claimed is:

1. A recombinant RNA molecule comprising a binding site specific for an RNA-directed RNA polymerase derived from a respiratory syncytial virus, operatively linked to a heterologous RNA sequence comprising the reverse complement of an mRNA coding sequence.

2. A recombinant RNA molecule comprising a heterologous RNA sequence comprising the reverse complement of an mRNA coding sequence, operatively linked to a 3'-noncoding viral sense flanking sequence of a respiratory syncytial virus containing the viral polymerase binding site, and to a 540 -noncoding viral sense flanking sequence of respiratory syncytial virus.

3. A recombinant RNP comprising the recombinant RNA molecule of claim 1 mixed with the purified RNA-directed RNA polymerase.

4. A recombinant RNP comprising the recombinant RNA molecule of claim 3 mixed with purified respiratory syncytial viral polymerase.

5. A recombinant RNP comprising the recombinant RNA molecule of claim 2 mixed with purified respiratory syncytial viral polymerase.

6. A chimeric virus comprising a respiratory syncytial virus containing a heterologous RNA sequence comprising the reverse complement of an mRNA coding sequence, operatively linked to a respiratory syncytial viral polymerase binding site.

7. A chimeric virus comprising a respiratory syncytial virus containing a heterologous RNA sequence comprising the reverse complement of an mRNA coding sequence, operatively linked to a polymerase binding site of the respiratory syncytial virus.

8. A method for producing a chimeric respiratory syncytial virus (RSV) comprising:

(a) culturing a host cell transfected with a heterologous RNA sequence comprising the reverse complement of an mRNA coding sequence operatively linked to a RSV

polymerase binding site and infected with a parental strain of RSV and,

(b) recovering said chimeric virus from the culture.

9. A chimeric RSV produced by the method of claim 8.

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L2: Entry 27 of 28 File: USPT Nov 26, 1996

US-PAT-NO: 5578473
DOCUMENT-IDENTIFIER: US 5578473 A

TITLE: Recombinant negative strand RNA virus

DATE-ISSUED: November 26, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Palese; Peter	Leonia	NJ		
Parvin; Jeffrey D.	Belmont	MA		
Krystal; Mark	Leonia	NJ		

US-CL-CURRENT: 435/235.1; 435/236, 435/320.1

CLAIMS:

What is claimed is:

1. A method for engineering a recombinant negative-strand RNA virus, comprising:

(a) transcribing a DNA molecule encoding a mutagenized gene of the negative-strand RNA virus operatively linked to the complement of the viral RNA-directed RNA polymerase binding site, to form a recombinant RNA molecule comprising the mutated viral gene operatively linked to the viral polymerase binding site, and combining the recombinant RNA molecule with the viral RNA-directed RNA polymerase to form a recombinant RNP;

(b) culturing a host cell transfected with the recombinant RNP and infected with a parental strain of the negative strand virus; and

(c) recovering chimeric viruses containing the mutated viral gene from the culture.

2. The method of claim 1 wherein the viral gene was mutagenized by site-directed mutagenesis.

3. A method for engineering a recombinant influenza virus, comprising:

(a) transcribing a DNA molecule encoding a mutagenized gene of the influenza virus operatively linked to the complement of the viral RNA-directed RNA polymerase binding site, to form a recombinant RNA molecule comprising the mutated influenza gene operatively linked to the viral polymerase binding site, and combining the recombinant RNA molecule with the viral RNA-directed RNA polymerase to form the recombinant RNP;

(b) culturing a host cell transfected with the recombinant RNP and infected with a parental strain of the influenza virus; and

(c) recovering chimeric influenza viruses containing the mutated viral gene from the culture.

4. The method of claim 3 wherein the influenza gene was mutagenized by site-directed mutagenesis.

5. The method of claim 34 wherein the mutated influenza gene is the NA gene of the influenza genome.

6. The method of claim 3 wherein the mutated influenza gene is the NS gene of the influenza genome.

7. The method of claim 3 wherein the mutated influenza gene is the HA gene of the influenza genome.

8. The method of claim 3 wherein the mutated influenza gene is the PB1 gene or the PB2 gene of the influenza genome.

9. The method of claim 3 wherein the mutated influenza gene is the PA gene of the influenza genome.

10. The method of claim 3 wherein the mutated influenza gene is the M gene of the influenza genome.

11. The method of claim 3 wherein the mutated influenza gene is the NP gene of the influenza genome.

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L2: Entry 28 of 28 File: USPT Nov 24, 1992

US-PAT-NO: 5166057
DOCUMENT-IDENTIFIER: US 5166057 A

TITLE: Recombinant negative strand RNA virus expression-systems

DATE-ISSUED: November 24, 1992

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Palese; Peter	Leonia	NJ		
Parvin; Jeffrey D.	Belmont	MA		
Krystal; Mark	Leonia	NJ		

US-CL-CURRENT: 435/69.1; 435/194, 435/235.1, 435/320.1, 435/463

CLAIMS:

What is claimed is:

1. A recombinant RNA molecule comprising a binding site specific for an RNA-directed RNA polymerase of a negative strand RNA virus, operatively linked to a heterologous RNA sequence comprising the reverse complement of an mRNA coding sequence.

2. The recombinant RNA molecule of claim 1 in which the polymerase binding site comprises the polymerase binding site contained in the 3'-noncoding flanking sequence of an influenza genome RNA segment.

3. The recombinant RNA molecule of claim 1 in which the polymerase binding site comprises the terminal 15 nucleotides of the 3'-terminus of an influenza genomic segment.

4. The recombinant RNA molecule of claim 2 in which the 3'-noncoding viral sense flanking sequence of influenza comprises the following sequence:

5'-CACCCUGCUUUUGCU-3'.

5. The recombinant RNA molecule of claim 2 in which the 3'-noncoding viral sense flanking sequence of influenza comprises the following sequence:

5'-CACCCUGCUUCUGCU-3'.

6. The recombinant RNA molecule of claim 2 in which the 3'-noncoding viral sense flanking sequence of influenza comprises the following sequence:

5'-CACCCUGUUUUUGCU-3'.

7. The recombinant RNA molecule of claim 2 in which the 3'-noncoding viral sense flanking sequence of influenza comprises the following sequence:

5'-CACCCUUGCUUUUGCU-3'.

8. A recombinant RNA molecule comprising a heterologous RNA sequence comprising the reverse complement of an mRNA coding sequence, operatively linked to a 3'-noncoding flanking sequence of an influenza vRNA containing the viral polymerase binding site, and to a 5'-noncoding flanking sequence of an influenza vRNA.

9. The recombinant RNA molecule of claim 8 in which the 5'-noncoding flanking sequence of an influenza vRNA comprises the first 22 nucleotides of the 5'-terminus of an influenza genomic segment.

10. The recombinant RNA molecule of claim 8 in which the 5'-noncoding flanking sequence of an influenza vRNA comprises the following sequence:

5'-AGUAGAAACAAGGGUGUUUUUU-3'.

11. A recombinant RNP comprising the recombinant RNA molecule of claim 1 mixed with the purified RNA-directed RNA polymerase.

12. A recombinant RNP comprising the recombinant RNA molecule of claim 2 mixed with purified influenza viral polymerase.

13. The recombinant RNP of claim 12 in which the influenza viral polymerase is obtained from RNPs fractionated by centrifugation on a CsCl gradient, in which the purified influenza viral polymerase is isolated from the region of the gradient correlating to 1.5 to 2.0 M CsCl.

14. A recombinant RNP comprising the recombinant RNA molecule of claim 8 mixed with purified influenza viral polymerase.

15. The recombinant RNP of claim 14 in which the influenza viral polymerase is obtained from RNPs fractionated by centrifugation on a CsCl gradient, in which the purified influenza viral polymerase is isolated from the region of the gradient correlating to 1.5 to 2.0 M CsCl.

16. A chimeric virus comprising a negative strand RNA virus containing a heterologous RNA sequence comprising the reverse complement of an mRNA coding sequence, operatively linked to a polymerase binding site of the negative-strand RNA virus.

17. A chimeric virus comprising influenza virus containing a heterologous RNA sequence comprising the reverse complement of an mRNA coding sequence, operatively linked to an influenza viral polymerase binding site.

18. The chimeric virus of claim 17 in which the heterologous RNA sequence is contained within segment 1 of influenza.

19. The chimeric virus of claim 17 in which the heterologous RNA sequence is contained within segment 2 of influenza.

20. The chimeric virus of claim 17 in which the heterologous RNA sequence is contained within segment 3 of influenza.

21. The chimeric virus of claim 17 in which the heterologous RNA sequence is contained within segment 4 of influenza.

22. The chimeric virus of claim 17 in which the heterologous RNA sequence is contained within segment 5 of influenza.

23. The chimeric virus of claim 17 in which the heterologous RNA sequence is contained within segment 6 of influenza.

24. The chimeric virus of claim 17 in which the heterologous RNA sequence is contained within segment 7 of influenza.

25. The chimeric virus of claim 17 in which the heterologous RNA sequence is contained within segment 8 of influenza.

26. A chimeric virus comprising influenza virus containing in addition to its eight genomic segments an additional RNA segment containing a heterologous RNA sequence comprising the reverse complement of an mRNA coding sequence, operatively linked to an influenza viral polymerase binding site.

27. A recombinant DNA molecule encoding the recombinant RNA molecule of claim 1 operatively linked to a transcription control element that binds a DNA-directed RNA polymerase.

28. A recombinant DNA molecule encoding the recombinant RNA molecule of claim 2 operatively linked to a transcription control element that binds a DNA-directed RNA polymerase.

29. A recombinant DNA molecule encoding the recombinant RNA molecule of claim 8 operatively linked to a transcription control element that binds a DNA-directed RNA polymerase.

30. A method for gene expression, comprising culturing a hose cell transfected with the recombinant RNP of claim 11 so that the heterologous gene is expressed in the culture.

31. A method for gene expression, comprising culturing a host cell transfected with the recombinant RNP of claim 12 so that the heterologous gene is expressed in the culture.

32. A method for gene expression, comprising culturing a host cell transfected with the recombinant RNP of claim 14 so that the heterologous gene is expressed in the culture.

33. A method for producing a chimeric negative-strand RNA virus, comprising culturing a host cell transfected with the recombinant RNP of claim 11 and infected with a parental strain of the negative strand RNA virus, and recovering the chimeric virus from the culture.

34. A method for producing a chimeric influenza virus, comprising culturing a host cell transfected with the recombinant RNP of claim 12 and infected with a parental strain of influenza, and recovering the chimeric influenza virus from the culture.

35. A method for producing a chimeric influenza virus, comprising culturing a host cell transfected with the recombinant RNP of claim 14 and infected with a parental strain of influenza, and recovering the chimeric influenza virus from the culture.

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L1: Entry 1 of 1 File: USPT Nov 24, 1998

US-PAT-NO: 5840520
DOCUMENT-IDENTIFIER: US 5840520 A

TITLE: Recombinant negative strand RNA virus expression systems

DATE-ISSUED: November 24, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Clarke; David Kirkwood	Pacifica	CA		
Palese; Peter M.	Leonia	NJ		

US-CL-CURRENT: 435/69.1; 424/199.1, 435/235.1, 435/320.1, 536/23.1

CLAIMS:

What is claimed is:

1. A recombinant RNA molecule comprising a binding site specific for an RNA-directed RNA polymerase derived from a respiratory syncytial virus, operatively linked to a heterologous RNA sequence comprising the reverse complement of an mRNA coding sequence.

2. A recombinant RNA molecule comprising a heterologous RNA sequence comprising the reverse complement of an mRNA coding sequence, operatively linked to a 3'-noncoding viral sense flanking sequence of a respiratory syncytial virus containing the viral polymerase binding site, and to a 540 -noncoding viral sense flanking sequence of respiratory syncytial virus.

3. A recombinant RNP comprising the recombinant RNA molecule of claim 1 mixed with the purified RNA-directed RNA polymerase.

4. A recombinant RNP comprising the recombinant RNA molecule of claim 3 mixed with purified respiratory syncytial viral polymerase.

5. A recombinant RNP comprising the recombinant RNA molecule of claim 2 mixed with purified respiratory syncytial viral polymerase.

6. A chimeric virus comprising a respiratory syncytial virus containing a heterologous RNA sequence comprising the reverse complement of an mRNA coding sequence, operatively linked to a respiratory syncytial viral polymerase binding site.

7. A chimeric virus comprising a respiratory syncytial virus containing a heterologous RNA sequence comprising the reverse complement of an mRNA coding sequence, operatively linked to a polymerase binding site of the respiratory syncytial virus.

8. A method for producing a chimeric respiratory syncytial virus (RSV) comprising:

(a) culturing a host cell transfected with a heterologous RNA sequence comprising the reverse complement of an mRNA coding sequence operatively linked to a RSV polymerase binding site and infected with a parental strain of RSV and,

(b) recovering said chimeric virus from the culture.

9. A chimeric RSV produced by the method of claim 8.

12. The vaccine of claim 1 or claim 2 in which the mRNA coding sequence encodes a foreign epitope.

13. The vaccine of claim 12 in which the foreign epitope comprises a fragment of the hepatitis B surface antigen.

14. The vaccine of claim 12 in which the foreign epitope comprises a fragment of a glycoprotein of a herpesvirus.

15. The vaccine of claim 12 in which the foreign epitope comprises a fragment of the VP1 protein of a poliovirus.

16. A composition comprising a chimeric negative strand RNA virus the genome of which contains the reverse complement of an mRNA coding sequence which encodes a human immunodeficiency virus epitope operatively linked to a polymerase binding site of the negative strand RNA virus, and a carrier.

17. The composition of claim 16 in which the human immunodeficiency virus epitope is a gp120 epitope.

L2: Entry 20 of 28 File: USPT Oct 13, 1998

US-PAT-NO: 5820871
DOCUMENT-IDENTIFIER: US 5820871 A

TITLE: Recombinant negative strand RNA virus expression systems and vaccines

DATE-ISSUED: October 13, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Palese; Peter	Leonia	NJ		
Garcia-Sastre; Adolfo	New York	NY		

US-CL-CURRENT: 424/209.1; 424/206.1, 435/320.1

CLAIMS:

What is claimed is:

1. A vaccine comprising a chimeric negative strand RNA virus the genome of which contains the reverse complement of an MRNA coding sequence operatively linked to a polymerase binding site of the negative strand RNA virus, and a pharmaceutically acceptable carrier.

2. The vaccine of claim 1 in which the chimeric negative strand RNA virus is a chimeric influenza virus.

3. The vaccine of claim 2 in which the mRNA coding sequence encodes a mutated influenza virus gene.

4. The vaccine of claim 3 in which the mutated influenza viral gene is a mutated PB2 gene.

5. The vaccine of claim 3 in which the mutated influenza viral gene is a mutated PB1 gene.

6. The vaccine of claim 3 in which the mutated influenza viral gene is a mutated PA gene.

7. The vaccine of claim 3 in which the mutated influenza viral gene is a mutated NP gene.

8. The vaccine of claim 3 in which the mutated influenza viral gene is a mutated HA gene.

9. The vaccine of claim 3 in which the mutated influenza viral gene is a mutated NA gene.

10. The vaccine of claim 3 in which the mutated influenza viral gene is a mutated NS gene.

11. The vaccine of claim 3 in which the mutated influenza viral gene is a mutated M gene.